Amdt. Dated August 22, 2006 Reply to Final Office Action of May 23, 2006

## Amendments to the Specification

between two nucleic acid sequences.

Please replace paragraph [0009] at page 3 with the following amended paragraph:

Please replace paragraph [0046] at page 14 with the following amended paragraph:

[0002] One known component of a CDK complex is a Cdc2 protein termed P34e662, which is required at both control points (G1-S and G2-M). Several other Cdc2 homologs have been isolated from human and plant species including yeast. One such yeast homolog is Cdc48, which plays a role in the spindle pole body separation in Saccharomyces cerevisiae. Another Cdc2 homolog has been described in Arabidopsis (Feiler et al. 1995 EMBO J 14:5626) that is highly expressed in the proliferating cells of the vegetative shoot, root, floral inflorescence and flowers and in rapidly growing cells. The Arabidopsis Cdc48 gene is up regulated in the developing microspores and ovules and down regulated in most differentiated cell types. In addition, this gene has been localized to the nucleus and during cytokinesis to the fragmoplast.

[0046] To determine the percent homology of two amino acid sequences (e.g., one of the sequences of SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9 and a mutant form thereof), the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The amino acid residues at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence (e.g., one of the sequences of SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9) is occupied by the same amino acid residue as at the corresponding position in the other sequence (e.g., a mutant form of the sequence selected from the polypeptide of SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9), then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The same type of comparison can be made

Please replace paragraph [0097] at page 31 with the following amended paragraph:

Appl. No. 10/768,511 Amdt. Dated August 22, 2006 Reply to Final Office Action of May 23, 2006

[0097] For such plant transformation, binary vectors such as pBinAR can be used (Höfgen and Willmitzer, 1990 Plant Science 66:221-230). Construction of the binary vectors can be performed by ligation of the cDNA in sense or antisense orientation into the T-DNA. 5prime to the cDNA a plant promoter activates transcription of the cDNA. A polyadenylation sequence is located 3-prime to the cDNA. Tissue-specific expression can be achieved by using a tissue specific promoter. For example, seed-specific expression can be achieved by cloning the napin or LeB4 or USP promoter 5-prime to the cDNA. Also, any other seed specific promoter element can be used. For constitutive expression within the whole plant, the CaMV 35S promoter can be used. The expressed protein can be targeted to a cellular compartment using a signal peptide, for example for plastids, mitochondria or endoplasmic reticulum (Kermode, 1996 Crit. Rev. Plant Sci. 4 (15):285-423). The signal peptide is cloned 5-prime in frame to the cDNA to archive subcellular localization of the fusion protein. Additionally, promoters that are responsive to abiotic stresses can be used with, such as the Arabidopsis promoter RD29A, the nucleic acid sequences disclosed herein. One skilled in the art will recognize that the promoter used should be operatively linked to the nucleic acid such that the promoter causes transcription of the nucleic acid which results in the synthesis of a an mRNA which encodes a polypertide. Alternatively, the RNA can be an antisense RNA for use in affecting subsequent expression of the same or another gene or genes.

Please replace paragraph [0129] at page 41 with the following amended paragraph:

[0129] Culturing was carried out in a climatic chamber at an air temperature of 25°C and light intensity of 55 micromols -1 m<sup>2</sup> (white light; Philips TL 65W/25 fluorescent tube) and a light/dark change of 16/8 hours. The moss was either modified in liquid culture using Knop medium according to Reski and Abel (1985, Planta 165:354-358) or cultured on Knop solid medium using 1% oxoid agar (Unipath, Basingstoke, England). The protonemas used for RNA and DNA isolation were cultured in aerated liquid cultures. The protonemas were comminuted every 9 days and transferred to fresh culture medium.

Please replace paragraph [0155] at page 53 with the following amended paragraph:

Amdt. Dated August 22, 2006 Reply to Final Office Action of May 23, 2006

[0155] T1 seeds were sterilized according to standard protocols (Xiong et al. 1999, Plant Molecular Biology Reporter 17: 159-170). Seeds were plated on ½ MS 0.6% agar supplemented

with 1% sucrose, 50  $\mu$ g/ml kanamycin (Sigma-Aldrich) and 2  $\mu$ g/ml benomyl (Sigma-Aldrich). Seeds on plates were vernalized for four days at 4°C. The seeds were germinated in a climatic

Seeds on plates were vernalized for four days at 4°C. The seeds were germinated in a climatic chamber at an air temperature of 22°C and light intensity of 40 micromols -1 m<sup>2</sup> micromol s<sup>-1</sup> m<sup>-2</sup>

(white light; Philips TL 65W/25 fluorescent tube) and 16 hours light and 8 hours dark day length

cycle. Transformed seedlings were selected after 14 days and transferred to ½ MS 0.6% agar

plates supplemented with 1% sucrose and allowed to recover for five-seven days.

Please replace paragraph [0156] at page 53 with the following amended paragraph:

[0156] T1 seedlings were transferred to dry, sterile filter paper in a petri dish and allowed to desiccate for two hours at 80% RH (relative humidity) in a Sanyo Growth Cabinet MLR-

350H,  $\frac{micromols}{1}$   $\frac{1m^2}{micromols}$  (white light; Philips TL 65W/25 fluorescent tube). The RH was then decreased to 60% and the seedlings were desiccated further for eight hours.

Seedlings were then removed and placed on  $\frac{1}{2}$  MS 0.6% agar plates supplemented with 2  $\mu$ g/ml benomyl and scored after five days.

Please replace paragraph [0157] at page 53 with the following amended paragraph:

[0157] Under drought stress conditions, PpCC-1 over-expressing Arabidopsis thaliana plants showed a 67% (6 survivors from 9 stressed plants) survival rate to the stress screening; PpCC-2, 75% (6 survivors from 8 stressed plants) and PpCC-3, 92% (11 survivors from 12 stressed plants), whereas the control only showed a an 11% survival rate (1 survivor from 9 stressed plants) (see Table 7). It is noteworthy that the analyses of these transgenic lines were performed with T1 plants, and therefore, the results will be better when a homozygous, strong expresser is found.

Please replace paragraph [0159] at page 54 with the following amended paragraph:

[0159] Seedlings were moved to petri dishes containing ½ MS 0.6% agar supplemented with 2% sucrose and 2 µg/ml benomyl. After four days, the seedlings were incubated at 4°C for 1

Andt. Dated August 22, 2006

Reply to Final Office Action of May 23, 2006

hour and then covered with shaved ice. The seedlings were then placed in an Environmental Specialist ES2000 Environmental Chamber and incubated for 3.5 hours beginning at -1.0°C decreasing -1°C hour 1°C per hour. The seedlings were then incubated at -5.0°C for 24 hours and then allowed to thaw at 5°C for 12 hours. The water was poured off and the seedlings were scored after 5 days.

Please replace paragraphs [0174]- [0177] at pages 58-59 with the following amended paragraphs:

[0174] The constructs pBPSSY032, pBPSsc335 and pBPSLVM186 were are used to

transform soybean as described below.

[0175] Seeds of soybean were <u>are</u> surface sterilized with 70% ethanol for 4 minutes at room temperature with continuous shaking, followed by 20% (v/v) Clorox supplemented with 0.05% (v/v) Tween for 20 minutes with continuous shaking. Then, the seeds were <u>are</u> rinsed 4 times with distilled water and placed on moistened sterile filter paper in a Petri dish at room temperature for 6 to 39 hours. The seed coats were <u>are</u> peeled off, and cotyledons are detached from the embryo axis. The embryo axis was <u>is</u> examined to make sure that the meristematic region is not damaged. The excised embryo axes were <u>are</u> collected in a half-open sterile Petri dish and air-dried to a moisture content less than 20% (fresh weight) in a sealed Petri dish until further use.

[0176] Agrobacterium tumefaciens culture was is prepared from a single colony in LB solid medium plus appropriate antibiotics (e.g. 100 mg/l streptomycin, 50 mg/l kanamycin) followed by growth of the single colony in liquid LB medium to an optical density at 600 nm of 0.8. Then, the bacteria culture was is pelleted at 7000 rpm for 7 minutes at room temperature, and resuspended in MS (Murashige and Skoog, 1962) medium supplemented with 100 µM acetosyringone. Bacterial cultures were are incubated in this pre-induction medium for 2 hours at room temperature before use. The axis axes of soybean zygotic seed embryos at approximately 15% moisture content were are imbibed for 2 hours at room temperature with the pre-induced Agrobacterium suspension culture. The embryos are removed from the imbibition culture and were are transferred to Petri dishes containing solid MS medium supplemented with 2% sucrose and incubated for 2 days, in the dark at room temperature. Alternatively, the embryos were are placed on top of moistened (liquid MS medium) sterile filter paper in a Petri

Amdt. Dated August 22, 2006 Reply to Final Office Action of May 23, 2006

12 hours photoperiod for about 80 days.

dish and incubated under the same conditions described above. After this period, the embryos were <u>are</u> transferred to either solid or liquid MS medium supplemented with 500 mg/L carbenicillin or 300mg/L cefotaxime to kill the agrobacteria. The liquid medium <u>was is</u> used to moisten the sterile filter paper. The embryos were <u>are</u> incubated <u>during for</u> 4 weeks at 25°C, under 150 µmol m<sup>2</sup>sec<sup>-1</sup> and 12 hours photoperiod. Once the seedlings produced roots, they were <u>are</u> transferred to sterile metromix soil. The medium of the *in vitro* plants <u>was is</u> washed off before <u>transferring</u> the plants <u>are transferred</u> to soil. The plants <u>were are</u> kept under a plastic cover for 1 week to favor the acclimatization process. Then the plants <u>were are</u> transferred to a growth room where they were are incubated at 25°C, under 150 µmol m<sup>-2</sup>sec<sup>-1</sup> light intensity and

[0177] The transgenic plants were are then screened for their improved drought, salt and/or cold tolerance according to the screening method described in Example 7 demonstrating to demonstrate that transgene expression confers stress tolerance.

Please replace paragraph [0178] at page 59 with the following amended paragraph:

[0178] The constructs pBPSSY032, pBPSsc335 and pBPSLVM186 were are used to transform rapseed rapeseed/canola as described below.

Please replace paragraph [0180]-[0182] at page 60 with the following amended paragraph:

[0180] The transgenic plants are then screened for their improved stress tolerance according to the screening method described in Example 7 demonstrating to demonstrate that transgene expression confers drought tolerance.

Please replace paragraph [0181] at page 60 with the following amended paragraphs:

[0181] The constructs pBPSSY032, pBPSsc335 and pBPSLVM186 were are used to transform corn as described below.

[0182] Transformation of maize (Zea Mays L.) is performed with the method described by Ishida et al. 1996. Nature Biotch 14745-50. Immature embryos are co-cultivated with Agrobacterium tumefaciens that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. This procedure provides a transformation efficiency of between 2.5%

Appl. No. 10/768,511 Docket No.: 15092-02

Amdt. Dated August 22, 2006 Reply to Final Office Action of May 23, 2006

and 20%. The transgenic plants are then screened for their improved drought, salt and/or cold tolerance according to the screening method described in Example 7 demonstrating to demonstrate that transgene expression confers stress tolerance.

Please replace paragraph [0183]-[0184] at page 60 with the following amended paragraphs:

[0183] The constructs pBPSSY032, pBPSsc335 and pBPSLVM186 were <u>are</u> used to transform wheat as described below.

[0184] Transformation of wheat is performed with the method described by Ishida et al. 1996 Nature Biotch. 14745-50. Immature embryos are co-cultivated with Agrobacterium tumefaciens that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. This procedure provides a transformation efficiency between 2.5% and 20%. The transgenic plants are then screened for their improved stress tolerance according to the screening method described in Example 7 demonstrating to demonstrate that transgene expression confers drought tolerance.